APPENDIX N
OHIO DISTRICT MICROBIOLOGY LABORATORY
RT-PCR ELUTION PROTOCOL

DAY ONE:

Step 1. Calibrate the pH meter using the 4.0, 7.0, and 10.0 buffer solutions. Sterilize the pH electrode by immersion into 0.525% bleach (10% household bleach) for 10 minutes, 2% thiosulfate for 1 minute, and a final rinse of sterile deionized water.

Step 2. Put on N-Dex gloves and wipe down the entire counter with 0.5% iodine and then 0.525% bleach.

Step 3. Attach sterile tubing (sterilized on inside and outside surfaces with 0.525% bleach for 30 minutes, dechlorinated with 0.05% sodium thiosulfate for several minutes, and rinsed thoroughly with sterile deionized water) to the outlet port of a sterile pressure vessel and to the inlet port of the cartridge housing module containing a 1-MDS filter to be tested.

Step 4. Place the sterile end of the tubing connected to the outlet port of the cartridge housing into a sterile 2-L beaker that contains a stir rod. Leave the sterile foil on top of the beaker around the tubing.

Step 5. Remove the top of the pressure vessel and pour 1,600 mL of 1.5% beef extract pH 9.5 (pre-warmed to room temperature) into the vessel. Replace the top and close the vent/relief valve.

Step 6. Turn on the air compressor by turning the red-handled switch to AUTO. Open the relief valve by turning the knob slowly to the right until you begin to hear air leaving the pressure tank. (The gauge above the relief valve shows the amount to pressure going through the hose, which should be close to 5 psi. The valve closest to the power switch shows the amount of pressure in the tank.) The pressure can be regulated two ways: the stopcock in the hose or the relief valve on the pressure vessel.

Step 7. Place gauze soaked with 0.5% iodine over the red button on the cartridge housing and hold down while slowly filling the housing with beef extract. Once the solution reaches the top of the filter and begins to flow through the outlet, turn off the pressure by opening the relief valve on the pressure vessel first and then closing the line valve. Allow the solution to come in contact with the 1-MDS filter for 1 minute.

Step 8. Increase the pressure to force the solution through the filter and collect the eluate into the sterile beaker.

Step 9. Turn off the pressure and disconnect the tubing that attaches the cartridge housing to the pressure vessel. Pour out the remaining liquid in the cartridge housing through the inlet into the beaker.

Step 10. Open the housing and add approximately 800 mL of a second aliquot of 1.5% beef extract (pour in slowly until the filter begins to float). Close the housing and allow to sit overnight at room temperature. Store the remaining 800 mL of beef extract in the refrigerator until the following day. Prewarm the beef extract before second day of use.

Step 11. Stir the eluate at a speed just sufficient to create a vortex. Add 1.6 g of analytical celite filter aid. Immediately adjust the pH to 4.0 with 1 M HCl (add the acid using the automatic buret drop by drop or it will inactive the viruses) and continue stirring for 10 minutes. Make sure to sterilize the pH electrode before and after each use by immersion into 0.525% bleach for 10 minutes, 2% thiosulfate for 1 minute, and a final rinse of sterile deionized water. (Change these solutions once a week.)

Step 12. Place a sterile 75-cm prefilter (rough side up) into a sterile Buchner funnel. Place the funnel onto a sterile 2-L filter flask which is attached to a vacuum source. Wet the filter with sterile water and then pull the eluate/celite mixture through the prefilter using vacuum. Place a large stir rod on the outside of the beaker to prevent the other stir rod from entering the funnel. Continuously swirl the solution while pouring into the funnel. If there is some remaining celite in the beaker, rinse with a small amount of sterile water and pour into the funnel.

Step 13. Transfer the funnel with the prefilter to a sterile 250-mL filter flask. Add 80 mL of 0.15 M sodium phosphate and allow to drip through by gravity. After the sodium phosphate has passed through the prefilter, apply a small vacuum to pull any residual solution through the filter. Discard the prefilter with celite into a biohazard bag to be autoclaved.

Step 14. Pour the sodium phosphate eluate into a sterile 150-mL beaker with a stir rod. Adjust the pH of the eluate to 7.3-7.4 with 1 M HCl (add the acid drop by drop) while stirring.

Step 15. Attach a sterile 60-mL syringe to a 0.22-μm Acrodisc sterilizing filter and pretreat by passing through 5 mL of 3% beef extract. (Disconnect the filter before removing the plunger of the syringe.)

Step 16. Pour half of the eluate into the syringe and then pass the eluate through the filter into a sterile 50-mL Corning centrifuge tube. Pour 20 mL of this into a separate sterile 50-mL centrifuge tube and label with "PCR-A," the date, the sample ID number, and the analyst's initials. Store this at 4°C until the following day.

Step 17. Continue filtering the eluate until you have 20 mL in the original centrifuge tube. Label this with "Tissue-A," the date, the sample ID number, and the analyst's initials. Store this at 4°C until the following day.

Step 18. The remaining eluate should be filtered into a sterile Nalgene bottle that is labeled with an "A," today's date, the sample ID number, and the analyst's initials. Store the Nalgene bottle at 4°C until the following day. Place the syringe filter and disposable syringe in a bag to be autoclaved.

Step 19. CLEAN UP: All dishes that have come in contact with the sample should be autoclaved for 30 minutes after the sample is processed to inactivate any viruses that may be present. They should then be washed with Liquinox, rinsed twice with deionized water, and autoclaved a second time. The 1-MDS filter should be autoclaved in a biohazard bag and then disposed of in the regular trash.

DAY TWO:

Step 20. Repeat Steps 1-3.

Step 21. Add the remaining portion of the second aliquot of beef extract to the pressure vessel. This should be connected to the cartridge housing that has been sitting overnight filled with 800 mL of beef extract and the test 1-MDS filter. Increase the pressure to force the solution through the filter and collect the eluate into a sterile 2-L beaker with a stir rod. Turn off the pressure at the source and disconnect the tubing. Repeat Steps 11-15.

Step 22. Pour half of the eluate into the syringe and then pass the eluate through the filter into a sterile 50-mL Corning centrifuge tube. Transfer 20 mL of the second-day eluate to the 50-mL centrifuge tube that contains the first PCR eluate. This tube should now contain 40 mL of eluate and should be labeled "PCR-A + B." Transfer 20 mL to the first-day tissue culture tube. This tube should now be labeled "Tissue-A + B."

Step 23. Place the remaining eluate into the Nalgene bottle that contains the first eluate and relabel with "A + B." All of the tubes and bottles should be stored at –70°C until further analysis.

Step 24. Repeat Step 19 for cleanup.